Letter to Sponsors Using Vero Cells as a Cell Substrate for Investigational Vaccines

Department of Health and Human Services
Public Health Service
Food and Drug Administration
1401 Rockville Pike
Rockville, MD 20852-1448

Division of Vaccines and
Related Products Applications
Telephone: (301) 827-3070

March 12, 2001

Dear:

The Center for Biologics Evaluation and Research (CBER) is issuing this letter to inform manufacturers of the following interim recommendations pertaining to viral vaccine products that are produced in Vero cel and investigated for human use. These recommendations are based on extensive internal discussions, consultation with outside experts, and comments received from the Vaccines and Related Biological Products Advisory Committee (VRBPAC) during the meeting held on May 12, 2000. In general, CBER currently views Vero cells as an acceptable cell substrate for viral vaccines, but has residual concerns sponsors should attempt to address.

CBER recommends that all products derived from Vero cells be free of residual intact Vero cells. If your manufacturing process does not include a validated filtration step or other validated procedure to clear residual intact Vero cells from the product, please incorporate such a procedure into your manufacturing process and submit the appropriate changes to your IND or MF.

Internal discussions and comments from the VRBPAC suggest the need for continued concern about the level of residual Vero cell DNA in products manufactured in these cells. Although the World Health Organization (WHO) currently accepts a limit of residual DNA from continuous cell lines of 10 ng per dos for these products when administered parenterally, CBER wishes to continue considering the level of ris posed by residual Vero cell DNA on a case-by-case basis for viral vaccines. Consideration will also be g to the method of vaccine administration, e.g., parenteral, mucosal, or other route. Based on this concern CBER recommends that you:

A. Measure the amount and size distribution of residual cellular DNA in your final product if you have done so already. Please submit these results to your IND or MF and describe them in terms of the amount of residual cellular DNA per human dose of final formulated vaccine.

B. Consider various methods (e.g., DNAse treatment) by which the amount and size of residual cellu DNA might be further reduced. Please comment on what you have done or intend to do to consid the introduction of additional DNA reducing methods into your process, as well as the potential im of such changes on the performance (e.g., immunogenicity) of the product.

Internal discussions and preliminary comments of the VRBPAC also suggest the need for tumorigenicity testing of each manufacturer's Vero master cell bank and the end-of-production-passage-level-cells (EO derived from this cell bank. The term "EOPC" is meant to include cells at the end of a production run, as as cells cultured from the master or working cell bank to a population doubling level comparable to or beyond cells at the end of production. EOPC should preferably be described in terms of population doublings from your Vero master cell bank. The preferred model for this test is the immunosuppressed newborn Wistar rat, which should be followed for a period of at least five months. Alternative tumorigenic models may also be appropriate in certain circumstances and their use should be discussed with CBER. any evidence of tumorigenic potential is demonstrated in these tests, or if the results are inconclusive, th need for additional tumorigenicity testing with cell lysates and/or DNA will also need to be discussed with CBER.

Please submit your responses to your IND(s) or MF(s) within six months from the date of issuance of thi letter. Please direct any questions in the interim to Dr. Rebecca Sheets at the telephone number above.

Sincerely yours,

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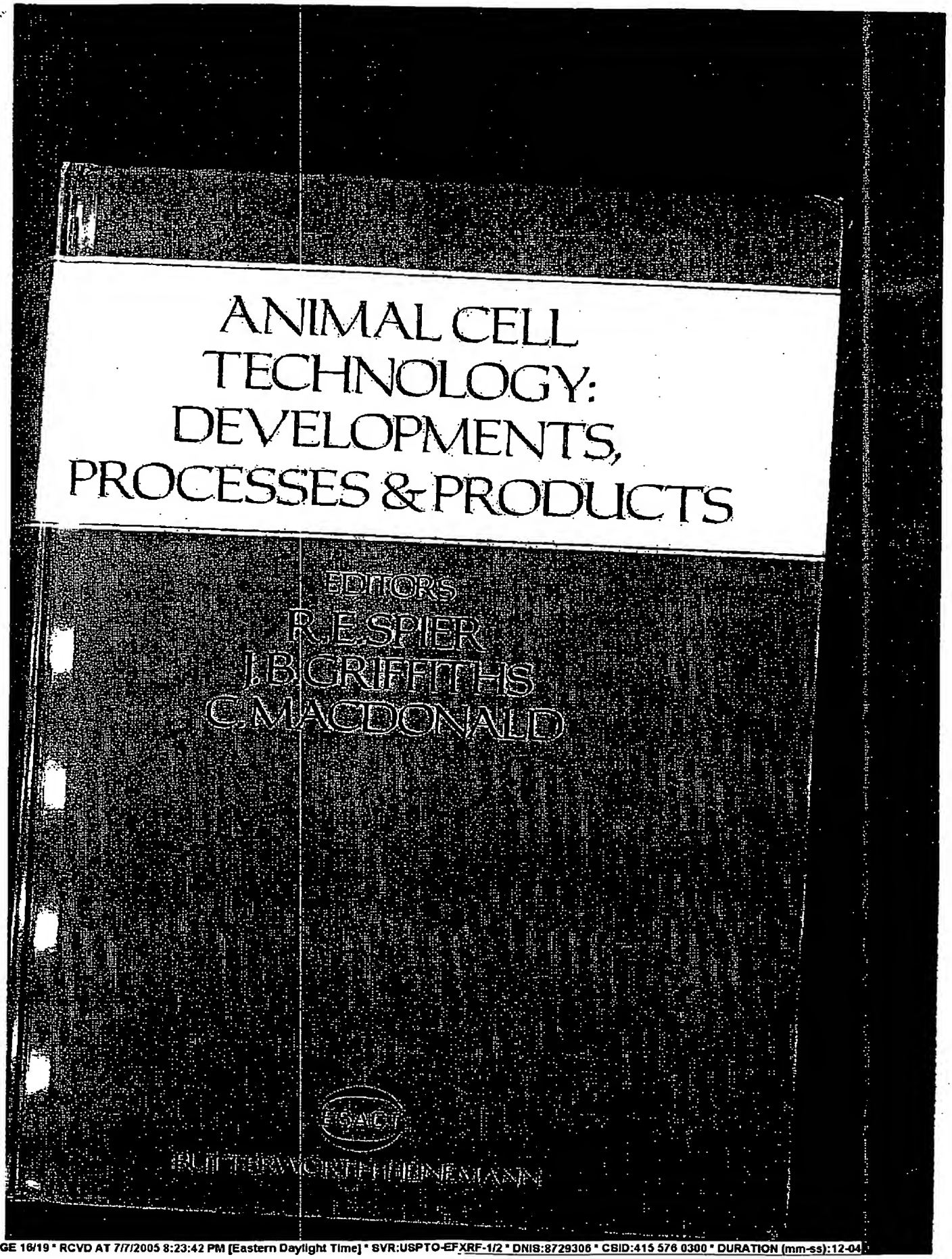
Karen Midthun, M.D.
Director
Office of Vaccines Research and Review
Center for Biologics Evaluation and Research

Updated May 20, 2002



QUANTITATION OF RESIDUAL DNA IN BIOLOGICAL PRODUCTS: NEW REGULATORY CONCERNS AND NEW METHODOLOGIES.

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ABSTRACT

The importance of residual DNA testing has been reinforced by two recent observations. First, the application of naked oncogenic DNA to mouse skin resulted in neoplastic transformation, second, intravenous injection of molecularly cloned proviral DNA of a simian immunodeficiency virus resulted in active viral infection. We have developed standardised procedures under GLP guidelines for the detection of residual DNA in biologicals which permit quantitation of contaminants to below 10pg per dose. The testing of samples in duplicate with the inclusion of two or more samples spiked with different levels of exogenous DNA is recommended. We have performed validation experiments which compare our hybridisation based assay with the biosensor-based Threshold™ system developed by the Molecular Devices Corporation. While the level of sensitivity of both assays is less than 10pg DNA, there are certain factors which should be considered in the selection of the assay, including the amount of protein to be evaluated, host species, and the availability of species-specific probes.

INTRODUCTION

The measurement of residual DNA in biological products is part of routine safety testing protocols. Potential problems associated with such DNA include malignant transformation of cells by activated oncogenes, uptake and subsequent expression of viral genomes in cells, and alteration of gene expression by insertion of sequences into sensitive control regions of genes. Risk is assessed on the quantity of DNA present and an arbitrary value of 10pg of residual DNA per therapeutic dose has been set as an acceptable level by regulatory authorities in Europe and the United States of America[1]. As well as amount, the risks are related to the size of the contaminating DNA.

While the alteration of gene expression by insertion of DNA into control regions is well documented, new evidence is accumulating that the risks from the first two events are more than theoretical. Naked plasmid DNA encoding the activated T24 H-*ras* was capable of transforming mouse endothelial cells *in vivo* after direct application of the DNA to scarified mouse skin[2]. Injection of molecularly cloned simian immunodeficiency virus (SIV_{MAC}) proviral DNA into susceptible monkeys led to an active infection of three out of four animals[3]. In both cases the amounts of DNA were at least ten-thousand fold greater than that found in purified product but they emphasise the potential risks associated with DNA

RESULTS AND DISCUSSION

DISTRIBUTION OF FRAGMENT SIZE

Purified residual DNA from a typical bulk harvest of final product from murine cells was examined by agarose gel-electrophoresis (Fig. 1). The majority of the DNA fragments were below 200 base pairs (bp) in size, distributed in bands of approximately 200bp, 120bp and 60bp, reflecting size selection during the initial purification process (Fig. 1A, b). In comparison, murine genomic DNA

digested to completion with Alu I, a frequent cutting restriction endonuclease, gave a visible smear of fragments ranging down from 2kbp on electrophoresis (Fig. 1A, c).

In order to detect the full size range of DNA fragments not visible by ethidium bromide staining, the DNA was transferred to a charged nylon membrane by capillary blotting and hybridised with ^{32}P-labelled murine DNA. Figure 1B shows the resulting autoradiogram obtained. Fragments over 6kbp were readily detected in the lanes containing the DNAs (Fig. 1B, c,d). The pattern of bands seen in the restriction endonuclease digested DNA was the result of hybridisation to banded repetitive sequences, the major radio-labelled component of the murine DNA probe.

It is evident from these data that although the majority of DNA fragments in residual DNA are too small to harbour complete open reading frames, larger fragments, capable of encoding functional proteins, are present. The size distribution of fragments of DNA present in final product will vary with the steps involved in the purification process.

SIZE PROFILE OF RESIDUAL DNA

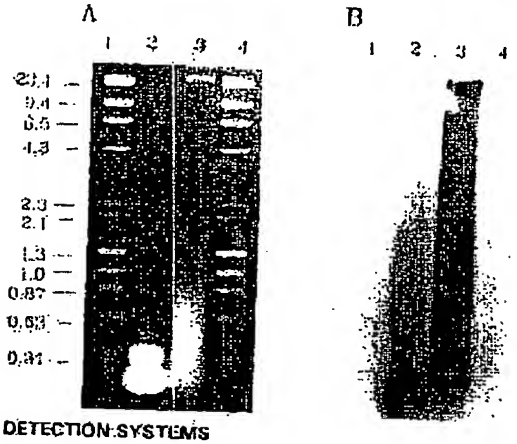

Figure 1. Electrophoresis of residual DNA. A, ethidium bromide stained. B, hybridized with murine probe.

DETECTION SYSTEMS

There are two main methods in current use for the quantitation of residual DNA in final products. First, by hybridisation where the DNA is purified from the test article, usually a protein, bound to a membrane and hybridised with an appropriate radioactively labelled probe[2]. The DNA level is evaluated by comparison of the test article signal with that of the controls from the autoradiogram. The controls should include an extraction of the test article or test article solute spiked with a known amount of DNA to allow an assessment of the efficiency of extraction. The extraction efficiency of DNAs from a test article is affected by a number of factors including: protein concentration, buffer composition (phosphate, EDTA, salts), the volume of the sample to be extracted and the size of the DNA fragments.

The Second method, the Molecular Devices Threshold™ system is a potentially more speedy, less labour intensive technique. The system uses two DNA binding proteins with high affinity for DNA but low sequence specificity. One protein is conjugated to an enzyme for signal generation and the other to a hapten for capture of DNA on a membrane. Quantitation is done by measuring enzyme activity through changes in surface potential on a silicon sensor.

Detailed results showing the validation and comparison of both methods will be presented elsewhere (Per and Sito). Briefly, recovery of DNA spiked into murine Mab, after pre-treated with proteinase K/SDS was high. The system also gave equivalent results to hybridization with respect to sensitivity and reproducibility. However, a comparison of the detection efficiency of the kit

control calf thymus DNA to murine, CHO and plasmid DNA revealed that although the Threshold assay was able to detect DNA from other species there was significant variation in the evaluation of DNA content of a series of control dilutions (Table 1).

TABLE 1 Comparison of detection of DNA from different species.

Results: pg detected for 100, 50, 25, 12.5, 6.3, and 3.1 pg DNA tested

Assay 1

Kit Calf Thymus		Mouse	CHO	Plasmid	Yeast tRNA
100	109.4	172.3	128.9	45.0	0.4
50 (#)	50	72.5	61.2	21.4	0.5
25	24.4	34.0	27.5	9.9	1.0
12.5	11.9	16.4	13.0	4.2	0.2
6.3	6.2	8.1	3.0	2.7	0.8
3.1	3.7	4.6	3.9	1.0	0

CONCLUSIONS

Before using the Threshold device careful validation and standardization for a particular situation are required. In particular the use of calf thymus DNA as standards would be misleading in the measurement of DNA in a test article and a validation of each species' DNA should be performed before measurements are made.

FUTURE

The use of semi-automated devices such as Threshold are valuable in routine testing where multiple identical samples are to be assessed once validated for a particular situation.

Testing of residual DNA for specific sequences is possible using polymerase chain reaction (PCR) assays, a useful safeguard where DNA of a known hazard, e.g. a virus, is likely to be present in a final product. Primers can be chosen such that they will only amplify sequences encoding a complete open reading frames and not small sheared fragments.

REFERENCES

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